APPENDIX E: TROUBLESHOOTING ALUQUANT

1 ALUQUANT® HUMAN QUANTITATION SYSTEM/ALUQUANT® CALCULATOR V3.0

1.1 Deleting Bad Data Points from the Standard Curve.

A minimum of 5 data points, including the water blank, are necessary for the standard curve. Occasionally, data points from the standard need to be deleted to reduce elevated background and/or to produce a more linear standard curve. The data points may be deleted, then the other standards and their RLU values must be copied and pasted into the cells so that the standard table is continuous. See Figures 1A and 1 B for an example. The Calculate button must be pressed again in order to generate a new extrapolated curve and to correct the DNA sample concentrations that may be off. Only the Project Coordinator or a supervisor may approve the deletion of data points. The deletion of data points is noted on the worksheet and initialed.

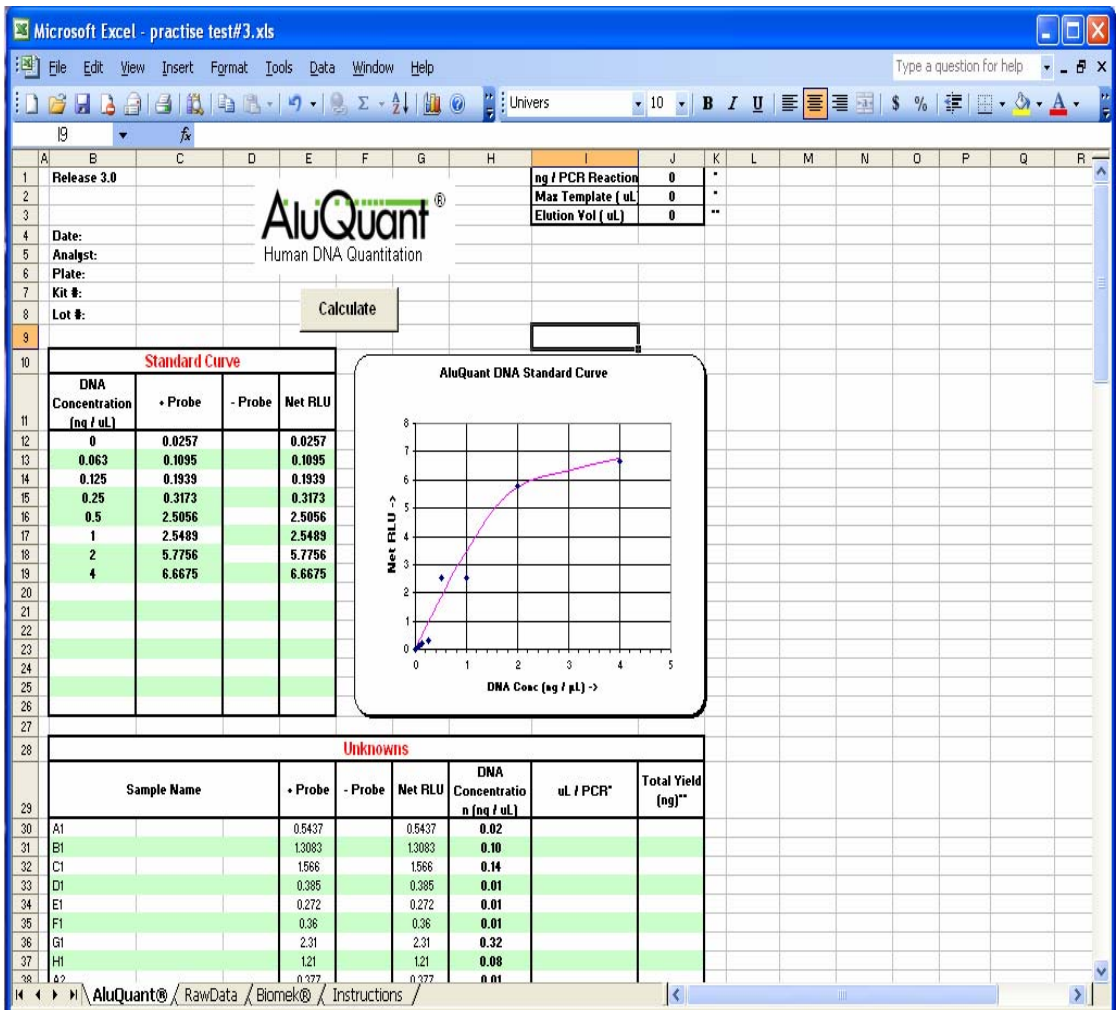

Figure 1A. Standard curve which may need data points deleted

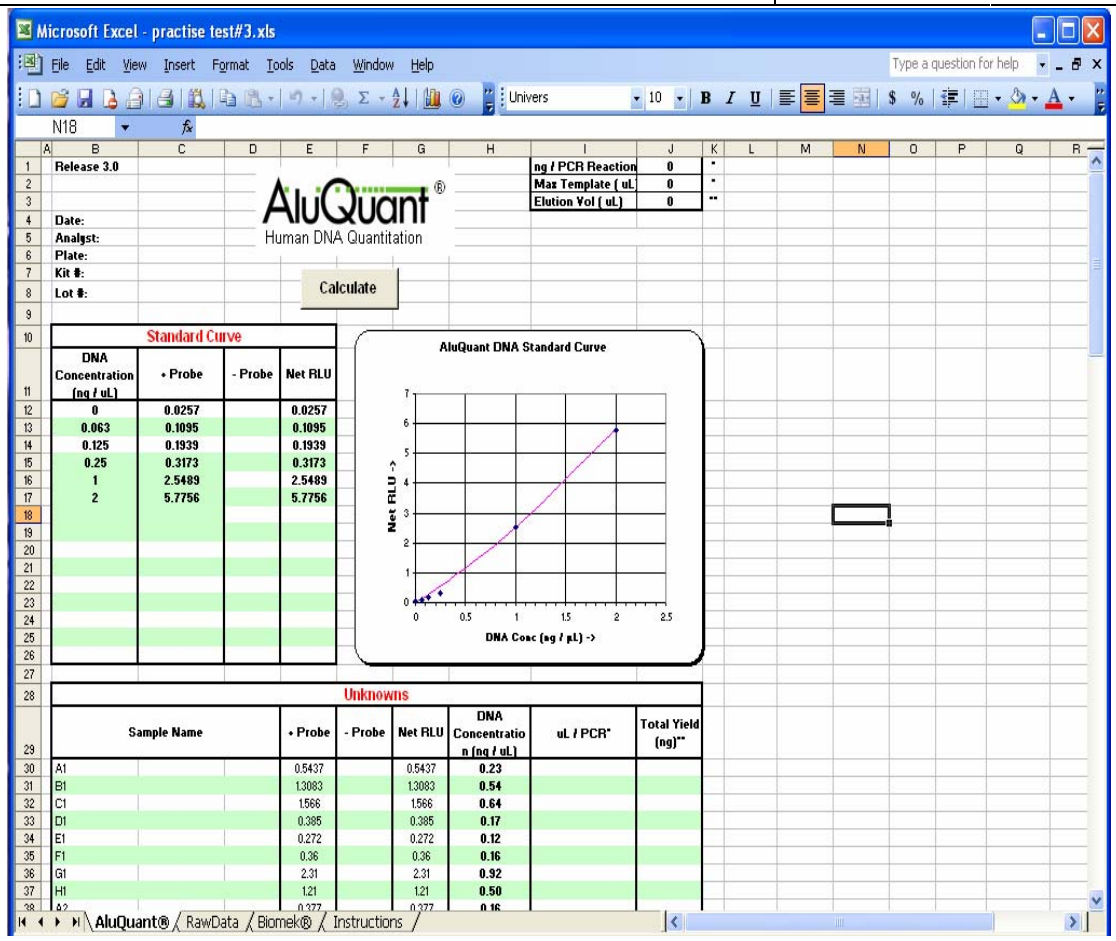

Figure 1B. Deletion of data points from standard curve

NOTE: Figures 1A and 1B illustrate the large difference in estimated DNA concentration for the samples when data points are deleted from the standard curve. Although AluQuant® Calculator v3.0 doesn't flag the user as to irregularities with the standard curve because the quadratic formula is capable of fitting any curve, it is imperative that the user evaluate the slope and character of the extrapolated (pink) curve to ensure the most accurate DNA concentration estimation. A large dip or even a flattening out in the RLU value for the 4 ng/µL standard will usually require that it be deleted since it can throw off the estimates for the most concentrated samples. The desired slope for the standard curve is approximately 45°.

1.2 Extremely Low RLU Values

It is normal for the RLU values to vary somewhat from run to run and they will usually halve when the L/L reagent is used that has been reconstituted and frozen. If the RLU values are not greater than 1.0-2.0 RLUs for the 4 ng/µL standard, even if the standards reduce in value proportionately with the lower concentrations as shown in Figure 2, it is likely that the luminometer lens is obscured. This can occur when the injector tip becomes clogged and mis-injects into the luminometer plate, hitting the top of the plate instead of into the wells. Another symptom of an obscured lens is the occasional large over estimate of a sample. This

is due to the fact that the lens is usually not uniformly obscured so that most of the signal may be muted, but an occasional sample signal may be detected at full strength and is thus vastly out of proportion with the muted standards.

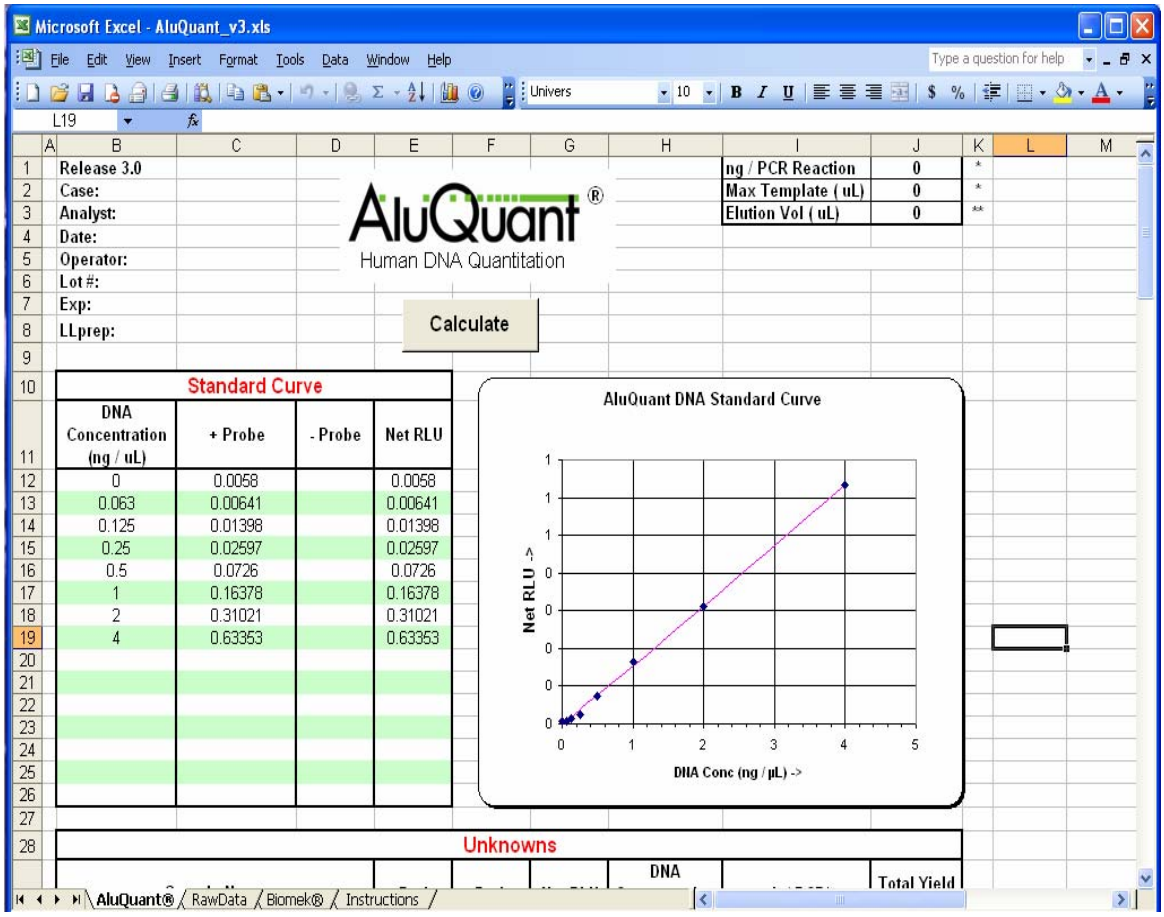

Figure 2. Abnormally low RLU values.

1.2.1 If extremely low RLU values are observed, the lens must be cleaned as described in Appendix E.

1.3 Inconsistent Quantitation Data.

1.3.1 Quantitation will become inconsistent if blockage of the injection tip occurs. Sometimes the data will become inconsistent before it is apparent that the injector tip is blocked. If inconsistent data is observed, then change the injection tip and test the instrument to determine if consistency has improved.

1.3.2 Quantitation will become inconsistent if the injector tubing becomes kinked. If replacing the injector tip fails to restore consistency to the quantitation data, then the injector tubing should be checked. The tubing is thin and can be easily bent, which

can possibly impede the injection of the full 50 µL aliquot of L/L reagent. New tubing should be used to replace the injector tubing by following the directions in the luminometer manual for replacing the injector tubing.

1.3.3 The Teflon seal of the plunger can also fail over time. Inconsistent data can also be a symptom that the plunger needs replacement. The plunger generally should be replaced every 3-5 years depending on usage. If replacing the tip and the tubing fails to rectify the issues with inconsistency, then the plunger may need replacement.

♦**END**